Variation in adult life history and stress resistance across five species of *Drosophila*

N. SHARMILA BHARATHI[1], N. G. PRASAD[1,3], MALLIKARJUN SHAKARAD[2] and AMITABH JOSHI[1]*

[1]*Evolutionary Biology Laboratory, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, P.O. Box 6436, Jakkur, Bangalore 560 064, India*
[2]*Behaviour, Ecology and Evolution Laboratory, Department of Biology, Poornaprajna Institute of Scientific Research, 4, Sadashivanagar, Bangalore 560 080, India*
[3]*Present address: Department of Biology, Queen's University, Kingston ON K7L 3N6, Canada*

Abstract

Dry weight at eclosion, adult lifespan, lifetime fecundity, lipid and carbohydrate content at eclosion, and starvation and desiccation resistance at eclosion were assayed on a long-term laboratory population of *Drosophila melanogaster*, and one recently wild-caught population each of four other species of *Drosophila*, two from the *melanogaster* and two from the *immigrans* species group. The relationships among trait means across the five species did not conform to expectations based on correlations among these traits inferred from selection studies on *D. melanogaster*. In particular, the expected positive relationships between fecundity and size/lipid content, lipid content and starvation resistance, carbohydrate (glycogen) content and desiccation resistance, and the expected negative relationship between lifespan and fecundity were not observed. Most traits were strongly positively correlated between sexes across species, except for fractional lipid content and starvation resistance per microgram lipid. For most traits, there was evidence for significant sexual dimorphism but the degree of dimorphism did not vary across species except in the case of adult lifespan, starvation resistance per microgram lipid, and desiccation resistance per microgram carbohydrate. Overall, *D. nasuta nasuta* and *D. sulfurigaster neonasuta* (*immigrans* group) were heavier at eclosion than the *melanogaster* group species, and tended to have somewhat higher absolute lipid content and starvation resistance. Yet, these two *immigrans* group species were shorter-lived and had lower average daily fecundity than the *melanogaster* group species. The smallest species, *D. malerkotliana* (*melanogaster* group), had relatively high daily fecundity, intermediate lifespan and high fractional lipid content, especially in females. *D. ananassae* (*melanogaster* group) had the highest absolute and fractional carbohydrate content, but its desiccation resistance per microgram carbohydrate was the lowest among the five species. In terms of overall performance, the laboratory population of *D. melanogaster* was clearly superior, under laboratory conditions, to the other four species if adult lifespan, lifetime fecundity, average daily fecundity, and absolute starvation and desiccation resistance are considered. This finding is contrary to several recent reports of substantially higher adult lifespan and stress resistance in recently wild-caught flies, relative to flies maintained for a long time in discrete-generation laboratory cultures. Possible explanations for these apparent anomalies are discussed in the context of the differing selection pressures likely to be experienced by *Drosophila* populations in laboratory versus wild environments.

[Sharmila Bharathi N., Prasad N. G., Shakarad M. and Joshi A. 2003 Variation in adult life history and stress resistance across five species of *Drosophila*. *J. Genet*. **82**, 191–205]

Introduction

Life-history evolution in *Drosophila* has been extensively studied, and laboratory selection studies on *D. melanogaster* have, in particular, yielded much insight into the various tradeoffs surrounding major life-history traits such as preadult development time, age-specific female fecundity and adult lifespan (reviewed in Prasad and Joshi 2003). Several studies support the notion of a cost of reproduction in terms of increased mortality and, hence, decreased adult lifespan in *D. melanogaster* (Rose 1984; Luckinbill and Clare 1985; Partridge and Andrews 1985; Chippindale *et al*. 1993, 1997; Zwaan *et al*. 1995; Cordts and Partridge 1996; Sgrò and Partridge 1999). The trade-

*For correspondence. E-mail: ajoshi@jncasr.ac.in.
This paper is dedicated to the memory of our friend and former colleague Dr Hans Raj Negi, who tragically passed away at a very young age in a road accident in November 2003.

Keywords. life-history evolution; lifespan; fecundity; starvation; desiccation; lipid; glycogen; sexual dimorphism; laboratory adaptation; *Drosophila*.

off between lifespan and fecundity is partly due to both traits sharing a resource, most likely lipid (Service 1987; Chippindale *et al*. 1993; Zwaan *et al*. 1995), and has been explained by a simple Y-model of resource allocation with the organism having to allocate limiting lipid reserves to either reproduction or somatic maintenance (van Noordwijk and de Jong 1986). Physiological studies, however, reveal that the tradeoff between lifespan and fecundity is not quantitatively exact (Djawdan *et al*. 1996; Simmons and Bradley 1997).

Further evidence indicating a major role for lipid and carbohydrate reserves in mediating adult life-history tradeoffs in *D. melanogaster* comes from the observations that (a) selection for increased lifespan results in correlated increases in starvation and desiccation resistance and lipid content (Service *et al*. 1985; Service 1987), (b) selection for increased starvation or desiccation resistance leads to a correlated increase in lifespan (Rose *et al*. 1992) and dry weight at eclosion (Chippindale *et al*. 1996, 1998), (c) selection for increased starvation resistance leads to increased lipid and carbohydrate content at eclosion (Chippindale *et al*. 1996; Djawdan *et al*. 1997), and (d) selection for increased desiccation resistance leads to increased carbohydrate but not lipid content at eclosion (Djawdan *et al*. 1997; Chippindale *et al*. 1998). Moreover, in these populations, selection for increased lifespan or starvation resistance did not result in correlated changes in metabolic rate, once the nonmetabolizing mass of lipid and carbohydrate was accounted for (Djawdan *et al*. 1997). However, all these results are from one laboratory and were done on sets of selected populations derived from a common long-term laboratory-reared ancestral population.

Selection for increased starvation or desiccation resistance in *D. melanogaster* in another set of studies using recently wild-caught flies yielded a somewhat different pattern of correlated responses to selection (Hoffmann and Parsons 1989a,b, 1993a,b; Harshman *et al*. 1999). In these studies, the evolution of increased desiccation resistance was accompanied by increases in starvation resistance (Hoffmann and Parsons 1989a) and lifespan (Hoffmann and Parsons 1993b), and decreases in overall activity level (Hoffmann and Parsons 1989a, 1993a) and mass-specific metabolic rate (Hoffmann and Parsons 1989a), though metabolic rates were not corrected for lipid/carbohydrate content (see discussion in Djawdan *et al*. 1997). Fractional lipid content, wet weight and body size did not differ between selected and control populations (Hoffmann and Parsons 1989a,b); carbohydrate content appears not to have been measured on these populations. Selection for increased starvation resistance led to significant correlated increases in weight, absolute lipid content and desiccation resistance, and a decrease in metabolic rate (Harshman *et al*. 1999). A marginally significant increase in carbohydrate content of starvation-selected populations was also observed (Harshman *et al*. 1999).

In addition to their importance to adult life history, starvation and desiccation resistance are also of interest because of their likely involvement in determining climatic adaptation and geographical distribution in *Drosophila* species (David *et al*. 1983; van Herrewege and David 1997; Hoffmann and Harshman 1999; Hoffmann *et al*. 2001a). In a study of 22 species of *Drosophila*, van Herrewege and David (1997) observed that temperate species, on average, were heavier, more resistant to starvation and desiccation, and had greater fractional lipid content than tropical species. Across the temperate species, starvation and desiccation resistance were significantly positively correlated, whereas they were uncorrelated among the tropical species. Similarly, fractional lipid content was significantly positively correlated with both starvation and desiccation resistance in the temperate, but not the tropical, species (van Herrewege and David 1997). On the other hand, opposite latitudinal clines for desiccation and starvation resistance have been observed in Indian populations of *D. kikkawai* (Karan and Parkash 1998), *D. ananassae*, *D. melanogaster* and *Zaprionus indianus* (Karan *et al*. 1998), with populations from lower latitudes (tropical) being relatively more starvation resistant and less desiccation resistant than those from higher latitudes (temperate, but with very hot and dry summers). However, clinal variation in starvation or desiccation resistance is not seen across *D. melanogaster* populations in South America (Robinson *et al*. 2000), or Europe and Africa (da Lage *et al*. 1990). In a study of *D. melanogaster* populations from the east coast of Australia, too, starvation and desiccation resistance did not exhibit strong latitudinal clines (Hoffmann *et al*. 2001a). Indeed, Hoffmann *et al*. (2001a) make the important point that although their data on population mean starvation and desiccation resistance could be interpreted as showing clinal variation, most of the variation in starvation and desiccation resistance in their study was actually found to be within populations, rather than among populations from different geographical regions. The comparison between variation within populations and variation among populations is, unfortunately, typically ignored in studies of clinal variation.

Overall, if one looks at studies of adult life history and starvation and desiccation resistance in *Drosophila*, there appears to be some disconnect between laboratory and field studies. Laboratory selection experiments have mostly been conducted only on *D. melanogaster* and it is, consequently, not clear whether the pattern of correlations among life-history and stress-resistance traits revealed by these studies is generally conserved across other *Drosophila* species as well, although it seems that at least some of the correlated responses to selection for increased desiccation resistance in an Australian population of *D. simulans* are qualitatively similar, albeit of much smaller magnitude, to those seen in a sympatric population of

D. melanogaster (Hoffmann and Parsons 1993a). Most studies on clinal variation in starvation and desiccation resistance, on the other hand, tend to ignore within-population variation and trait associations, and often do not include assays of life-history traits. As noted earlier, patterns of correlated responses to selection on starvation and desiccation resistance in *D. melanogaster* have been seen to differ between sets of studies using different starting material, and it has been suggested that long-term laboratory populations, adapted to living in a benign and nutrient-rich environment, may exhibit different underlying physiological mechanisms of evolved stress resistance than wild-caught populations, and that this difference may partly explain the observed discrepancy between selection studies (Harshman and Hoffmann 2000). It has been shown recently that populations of *D. melanogaster* reared in the laboratory on short, discrete-generation regimes undergo a fairly rapid decline in starvation and desiccation resistance (Hoffmann *et al*. 2001b), and in adult lifespan (Sgrò and Partridge 2000). In another study, it was shown that adult lifespan of a recently wild-caught strain of *D. melanogaster* was at par with that of laboratory strains successfully selected for increased lifespan, and significantly greater than laboratory strains maintained on a two-week discrete-generation cycle (Linnen *et al*. 2001). These findings suggest that long-term laboratory cultures maintained on short, discrete-generation regimes are likely to have high early-life fecundity, low lifespan, and low starvation and desiccation resistance, compared to wild populations, and that they may, consequently, respond to selection for increased lifespan or stress resistance via physiological mechanisms different from those that would be seen in wild populations subjected to the same selection pressure (Sgrò and Partridge 2000; Linnen *et al*. 2001; Hoffmann *et al*. 2001b).

It is against this backdrop—a combination of often contrasting results from studies on stress resistance and life history in *Drosophila* species, and an almost exclusive focus on *D. melanogaster* in studies of within-species trait correlations and life history tradeoffs—that we have initiated what is envisaged to be a series of long-term studies of life-history evolution in Indian species of *Drosophila*, other than *D. melanogaster*, in which a combination of laboratory experiments and field studies will be used in an attempt to understand patterns of variation and covariation within and among species for traits related to life history, in the context of selection pressures likely to be operating in nature on these populations. While there have been a few studies of stress resistance in Indian drosophilid species (mostly from the *melanogaster* species group), there have been practically no systematic studies of life-history variation in conjunction with stress resistance in Indian drosophilids. In this paper, we report preliminary results from a survey of adult life-history and stress-resistance related traits in one recently wild-caught southern Indian population each of four *Drosophila* species—two each from the *melanogaster* and *immigrans* species groups—along with a long-term laboratory population of *D. melanogaster* as a standard for comparison. While these results are preliminary, and do not conclusively support or exclude any particular hypothesis about the relationship between life-history and stress-resistance traits, they are, nevertheless, instructive in that they suggest that the contrast between laboratory and wild populations, and patterns of trait relationships across species, may be more variable than often thought.

Materials and methods

***Experimental populations*:** One population each of five species of *Drosophila* were used in this study. Three of the species belonged to the subgenus *Sophophora*, species group *melanogaster*, namely *D. melanogaster* (subgroup *melanogaster*), *D. ananassae* (subgroup *ananassae*, species complex *ananassae*), and *D. malerkotliana* (subgroup *ananassae*, species complex *bipectinata*). The other two species belonged to the subgenus *Drosophila*, species group *immigrans*, subgroup *nasuta*, namely *D. nasuta nasuta* (frontal sheen complex) and *D. sulfurigaster neonasuta* (orbital sheen complex). The *D. melanogaster* population was one of the JB populations (JB-1) previously described in detail (Sheeba *et al*. 1998) and has been in the laboratory for over 700 generations. The populations of *D. ananassae*, *D. malerkotliana* and *D. n. nasuta* were collected from orchards and domestic garbage dumps in different parts of Bangalore, whereas *D. s. neonasuta* was collected at both Bangalore and Mysore, India. At the time of this study, the *D. ananassae* population had been through 12 generations in the laboratory, whereas the populations of *D. malerkotliana*, *D. n. nasuta* and *D. s. neonasuta* had been in the laboratory for two, three and one generation, respectively. The *D. ananassae* population was initiated from ~300 inseminated females, collected during May–June 2001, while the other three populations were established from about 70 inseminated females each, collected during October–November 2001. Collection of flies was done by a combination of banana traps and net sweeping, mostly during early morning and dusk.

The *D. melanogaster* population has been in the laboratory for more than 700 generations on a 21-day discrete-generation cycle at 25°C, under constant light and ~90% relative humidity, on banana–jaggery food. The preadult stages are maintained at a moderate density of about 60–80 eggs per vial (9 cm × 2.4 cm). Eclosed adults are transferred to a plexiglas cage (25 cm × 20 cm × 15 cm) on the eighteenth day from egg lay, and are given food medium supplemented with live yeast paste for about two and a half days prior to egg collection for the next generation. The populations of the other four species were

also maintained in the laboratory under the same conditions, except that cornmeal, rather than banana–jaggery, food was used, and the number of breeding adults was about 1200 flies per population, compared to ~1800 in the case of the *D. melanogaster* population. Moreover, since the lifespan and fecundity of the wild-caught species were relatively low (see Results), these four populations were maintained on a discrete generation of less than 21 days. Adults were collected into cages as they eclosed, provided with cornmeal food supplemented with live yeast paste, and eggs were collected about two days after the last eclosions had occurred, which would be about five to six days after the first eclosions. All populations were assayed on the food medium on which they were routinely reared.

***Dry weight assay*:** Freshly eclosed adults developing in vials set up at a density of about 50 eggs per vial were collected within 2 h of eclosion, killed by freezing, dried for 36 h at 70°C, and weighed in batches of five males or five females. Five batches each of males and females were weighed for each species.

***Adult lifespan assay*:** Freshly eclosed adults from vials set up at a density of about 50 eggs per vial were collected, and virgin flies of both sexes were segregated within 6 h of eclosion. Separate assays were done to examine the lifespan of reproducing and virgin flies of each species. Adult lifespan of virgins was assessed by setting up 20 vials per species containing either eight males or eight females (10 vials per sex). In the case of reproducing flies, 20 vials were set up for each species, with each vial containing four males and four females. Flies were transferred to fresh food vials every alternate day and mortality was recorded daily; any flies dying were not replaced during the course of the assay, which continued until all flies had died. For reproducing flies, the degree of sexual dimorphism in lifespan was also calculated, using vial means for males and females, as the difference between male and female lifespan, divided by average lifespan across sexes.

***Lifetime fecundity assay*:** Adults eclosing in vials set up at a density of about 50 eggs per vial were collected within 6 h of eclosion, and distributed into fresh vials, with each vial containing one male and one female. For each species, 40 such vials were set up. The flies were transferred to fresh vials every day, and the number of eggs laid by each female during the preceding 24 h was recorded. The assay was continued until all females died. Dead females were not replaced during the course of the assay, whereas dead males were replaced from a cohort of the same age that was being maintained in parallel. In addition to recording the total number of eggs laid by a female over her lifetime (total lifetime fecundity), the average daily fecundity was also calculated by dividing the total lifetime fecundity by the number of days the female lived.

***Lipid content assay*:** Adults eclosing in vials set up at a density of about 50 eggs per vial were collected within 2 h of eclosion, and killed by freezing, dried for 36 h at 70°C and weighed in batches of either five males or five females to obtain dry weight prior to lipid extraction. After weighing, the flies of each batch were kept in 1.5 ml of diethyl ether and gently agitated on a gel rocker for 24 h. The ether was then removed, and the flies were again dried for 12 h at 70°C and weighed. The difference in dry weight of the flies in each batch before and after lipid extraction, divided by the number of flies (5), was taken as the absolute lipid content per fly. This procedure is similar to that of Zwaan *et al.* (1991), with minor modifications. For each batch, fractional lipid content was also calculated by dividing the absolute lipid content of the batch by the dry weight of the batch before lipid extraction.

***Starvation resistance assay*:** Adults eclosing in vials set up at a density of about 50 eggs per vial were collected within 2 h of eclosion, and distributed in batches of either five males or five females into vials containing non-nutritive agar (12.4 g agar and 2.4 g *p*-hydroxybenzoic acid in 23 ml ethanol per litre). Five such vials each of males and females were set up per species. The vials were observed every two hours for mortality, and the assay continued until all flies had died. The time till death in hours for each fly was taken as the absolute starvation resistance. The procedure is similar to that of Zwaan *et al.* (1991) with minor modifications. The time to death in hours divided by the mean absolute lipid content for the species was also calculated, as it can be interpreted as reflecting the degree to which lipid reserves have not been committed to fecundity in females and are, therefore, available for somatic maintenance (e.g. see discussion in Zwaan *et al.* 1991; Leroi *et al.* 1994; Chippindale *et al.* 1996).

***Carbohydrate content assay*:** Adults eclosing in vials set up at a density of about 50 eggs per vial were collected within 2 h of eclosion, killed by freezing, dried for 36 h at 70°C, and weighed in batches of either five males or five females to obtain dry weight prior to carbohydrate estimation, which was done following the procedure in Djawdan *et al.* (1997) with minor modifications. The flies in each batch were then crushed in a small volume of distilled water, and the final volume was made up to 1 ml. These samples were then incubated in a water bath at 90°C for 30 min. After incubation, 500 µl of the supernatant in each sample was taken and 4 ml of anthrone reagent added to it, followed by thorough mixing on a vortex mixer. The samples were then heated in a boiling water bath for

30 min, and allowed to cool to room temperature. The optical density of each sample was then measured in a colorimeter at 620 nm, and the total glucose content of the flies in each batch was estimated from a glucose standard graph constructed for the assay. For each batch, fractional carbohydrate content was also calculated by dividing the absolute carbohydrate content of the batch by the dry weight of the batch before carbohydrate extraction.

***Desiccation resistance assay*:** Adults eclosing in vials set up at a density of about 50 eggs per vial were collected within 2 h of eclosion, and distributed in batches of either five males or five females into empty vials plugged with cotton wrapped around a desiccant ($CaCl_2$). Five such vials each of males and females were set up per species, and the vials were placed in a desiccator. The vials were observed every hour for mortality, and the assay continued until all flies had died. The time till death in hours for each fly was taken as the absolute desiccation resistance. The time to death in hours divided by the mean absolute carbohydrate content for the species was also calculated.

***Statistical analyses*:** All analyses were performed using STATISTICA™ for Windows Release 5.0 B (StatSoft, Inc. 1995). Data for all traits except fecundity were subjected to two-way analyses of variance (ANOVA) with sex and species treated as fixed factors, and batch or vial means used as input data for the analysis. In the case of lifetime and daily fecundity, data on individual females were subjected to one-way ANOVA to look for species differences. All pairwise multiple comparisons were done using Tukey's HSD test, with the appropriate modification for unequal sample sizes where necessary. The degree of sexual dimorphism for lifespan in reproducing flies was subjected to one-way ANOVA with species as the sole factor.

Results

Dry weight at eclosion

Dry weights at eclosion varied significantly across species, *D. s. neonasuta* being the heaviest and *D. malerkotliana* the lightest (figure 1). The range of variation was quite large, *D. s. neonasuta* flies being almost thrice as heavy as *D. malerkotliana* flies. The ANOVA revealed significant main effects of species ($F_{4,40} = 539$, $P < 0.001$), sex ($F_{1,40} = 369$, $P < 0.001$) and a significant interaction between species and sex ($F_{4,40} = 11$, $P < 0.001$). Females were significantly heavier than males in each species (figure 1).

Adult lifespan

Overall, both reproducing and virgin flies from the *melanogaster* group species had greater adult lifespan than those from the two *immigrans* group species (figure 2), and the ANOVA revealed significant effects of species and the species × sex interaction on the lifespan of both reproducing and virgin flies, whereas the main effect of sex was significant only in the case of reproducing flies (table 1). The degree of sexual dimorphism in lifespan varied significantly across species in reproducing flies (one-way ANOVA: $F_{4,95} = 8.62$, $P < 0.001$), being highest in the long-term laboratory population of *D. melanogaster* (table 2). The general tendency was for males to outlive females in the three *melanogaster* group species, and for females to outlive males in the two *immigrans* group species, although the only substantial and significant difference between male and female lifespan was seen in *D. melanogaster* (figure 2). Virgin flies lived significantly longer than reproducing flies only in the case of *D. melanogaster*; in the four recently wild-caught species, the lifespans of virgin and reproducing flies did not differ significantly (figure 2).

Lifetime fecundity

Mean total lifetime fecundity varied significantly across species (one-way ANOVA: $F_{4,195} = 51.79$, $P < 0.001$), *D. melanogaster* being the most fecund, followed by *D. ananassae* and *D. malerkotliana*, and finally by *D. n. nasuta* and *D. s. neonasuta* (figure 3). The ordering of species means for total lifetime fecundity was similar to that seen in the case of lifespan of reproducing flies (figure 2), raising the possibility that lifetime fecundity dif-

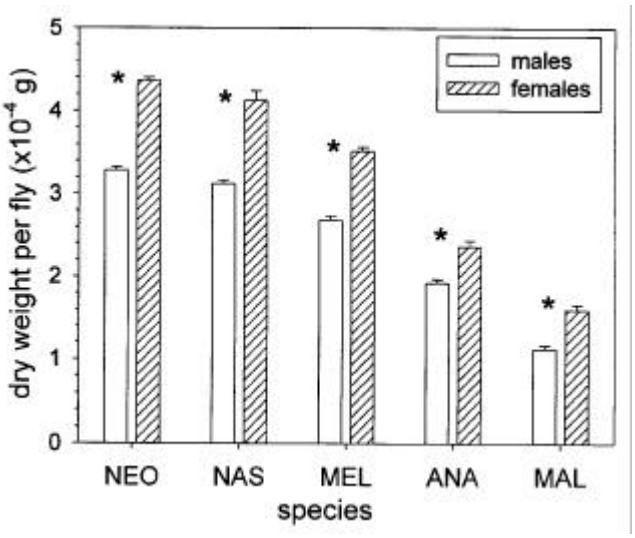

Figure 1. Mean (± SE) dry weight (in 10^{-4} g per fly) at eclosion of males and females from the five species (NEO, *D. s. neonasuta*; NAS, *D. n. nasuta*; MEL, *D. melanogaster*; ANA, *D. ananassae*; MAL, *D. malerkotliana*). All pairwise differences between species means were significant. Asterisks denote significant differences between mean male and female weight within each species. Significance at the 0.05 level was determined for each multiple comparison by Tukey's HSD test.

ferences among species could be largely due to lifespan differences rather than differences in daily egg production. However, average daily fecundity also varied significantly across species (one-way ANOVA: $F_{4,195} = 25.75$, $P < 0.01$), the only significant pairwise differences being those between *D. melanogaster* and all other species, and between *D. malerkotliana* and *D. s. neonasuta* (figure 3). The long-term laboratory population of *D. melanogaster* was clearly far more fecund than any of the other four species. Interestingly, even among just the four recently wild-caught species, *melanogaster* group species tended to be lighter in weight, longer-lived and more fecund than the larger *immigrans* group species, a pattern at odds with that of within-species correlations among weight, fecundity and lifespan revealed by studies on laboratory populations of *D. melanogaster* (e.g. Partridge and Fowler 1992, 1993; Hillesheim and Stearns 1992).

Lipid content

Both absolute and fractional lipid content varied significantly across species, with significant ANOVA main effects of species and sex, but no significant species × sex interaction, for both traits (table 3). Mean absolute lipid content of females (0.668×10^{-4} g) was significantly greater than that of males (0.424×10^{-4} g). Females also had significantly higher mean fractional lipid content (0.225) than males (0.178). Mean absolute lipid content of *D. s. neonasuta* was significantly greater (Tukey's HSD test, $P < 0.001$) than the means of the other four species, which did not differ significantly among themselves (figure 4). With regard to fractional lipid content, too, the species clustered into two groups within which species means did not significantly differ. Overall, *D. ananassae*, *D. malerkotliana* and *D. s. neonasuta* constituted the group with higher mean fractional lipid content whereas *D. melanogaster* and *D. n. nasuta* had similar, and relatively lower, fractional lipid levels (figure 4).

Table 1. Summary of results from two separate analyses of variance carried out on adult lifespan of reproducing and virgin males and females in the five species.

	Reproducing flies		Virgin flies	
Effect (d.f.)	*F*	*P*	*F*	*P*
Species (4)	154.64	< 0.001	193.45	< 0.001
Sex (1)	4.27	0.040	1.10	0.296
Species × sex (4)	8.44	< 0.001	3.15	0.018

Error d.f. were 190 and 89 for reproducing and virgin flies, respectively.

Table 2. Mean (± SE) degree of sexual dimorphism for adult lifespan in reproducing flies of the five species.

Species	Reproducing flies	
D. melanogaster	0.244^{a}	± 0.072
D. malerkotliana	0.148^{ab}	± 0.096
D. ananassae	0.054^{ab}	± 0.046
D. n. nasuta	-0.129^{bc}	± 0.087
D. s. neonasuta	-0.290^{c}	± 0.053

Means with the same alphabetical superscripts were not significantly different at the 0.05 level in pairwise comparisons, using Tukey's HSD test.

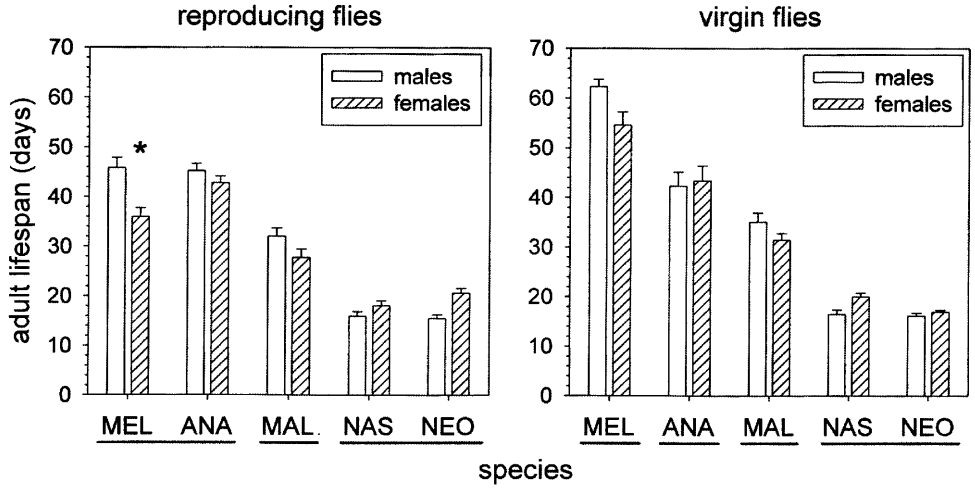

Figure 2. Mean (± SE) adult lifespan of reproducing and virgin males and females from the five species. Horizontal lines below the X-axes connect species whose means did not significantly differ among themselves. Asterisks denote significant differences between mean male and female lifespans within each species × reproductive status combination. Significance at the 0.05 level was determined for each multiple comparison by Tukey's HSD test.

Starvation resistance

Both absolute starvation resistance and starvation resistance per microgram lipid varied significantly across species, with significant ANOVA main effects of species and sex (table 3). The species × sex interaction, however, was significant only for starvation resistance per microgram lipid (table 3). Mean absolute starvation resistance of females (92.32 h) was significantly greater than that of males (82.17 h), whereas mean starvation resistance per microgram lipid in males (2.18 h/μg) was significantly higher than in females (1.43 h/μg) (figure 5). Overall, averaged across sexes, mean absolute starvation resistance of *D. malerkotliana*, the species with the least absolute lipid content (figure 4), was significantly lower than that of all other species, whereas the means for absolute starvation resistance of *D. melanogaster*, *D. n. nasuta* and *D. s. neonasuta* did not differ significantly among themselves (figure 5). Mean absolute starvation resistance of *D. ananassae* was intermediate between that of *D. malerkotliana* on the one hand, and those of *D. melanogaster*, *D. n. nasuta* and *D. s. neonasuta* on the other (figure 5).

Starvation resistance per microgram lipid varied across a greater range in males compared to females, and the pattern of differences among species also varied significantly with sex (table 3, figure 5). In females, the only significant pairwise differences (Tukey's HSD test, $P < 0.05$) in starvation resistance per microgram lipid between species were those between *D. s. neonasuta* and the other four species (figure 5). In males, on the other hand, species clustered into three groups based on the significance of pairwise comparisons for starvation resistance per microgram lipid. The highest mean starvation resistance per microgram lipid was seen in *D. malerkotliana* and *D. melanogaster*, the lowest in the two *immigrans* group species, and an intermediate value in *D. ananassae* (figure 5).

Carbohydrate content

Carbohydrate content of flies is typically used as a surrogate measure of the amount of glycogen in the flies (Graves *et al*. 1992; Chippindale *et al*. 1998; Hoffmann and Harshman 1999; Harshman and Hoffmann 2000). Both absolute

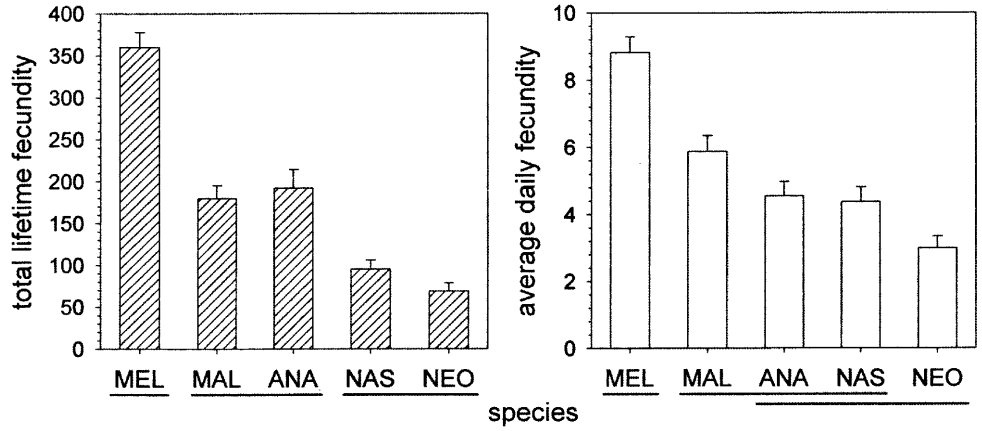

Figure 3. Mean (± SE) number of eggs laid by females from the five species over their lifetime, and over one day on average. Horizontal lines below the X-axes connect species whose means did not significantly differ among themselves. Significance at the 0.05 level was determined for each multiple comparison by Tukey's HSD test.

Table 3. Summary of results from four separate analyses of variance carried out on absolute lipid content, fractional lipid content, absolute starvation resistance and starvation resistance per microgram lipid in males and females from the five species.

	Absolute lipid content		Fractional lipid content		Absolute starvation resistance		Starvation resistance per μg lipid	
Effect (d.f.)	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species (4)	17.86	< 0.001	8.86	< 0.001	26.34	< 0.001	81.67	< 0.001
Sex (1)	42.43	< 0.001	9.96	0.004	17.43	< 0.001	254.07	< 0.001
Species × sex (4)	0.42	0.790	1.93	0.128	1.57	0.120	29.18	< 0.001

Error d.f. were 34 and 40 for the ANOVAs on lipid content and starvation resistance, respectively.

and fractional carbohydrate content varied significantly across species, with significant ANOVA main effects of species and sex, but no significant species × sex interaction, for both traits (table 4). Mean absolute carbohydrate content of females (28.14×10^{-6} g) was significantly greater than that of males (18.10×10^{-6} g). Females also had marginally significantly higher mean fractional carbohydrate content (0.096) than males (0.080). Overall, *D. ananassae* showed the highest absolute and fractional carbohydrate content (figure 6). Averaged across sexes, the species clustered into two groups based on significant differences in mean absolute carbohydrate content. *D. ananassae*, *D. n. nasuta* and *D. s. neonasuta* had relatively higher mean absolute carbohydrate content than *D. melanogaster* and *D. malerkotliana* (figure 6). In the case of fractional carbohydrate content, *D. melanogaster*, *D. n. nasuta* and *D. s. neonasuta* clustered together with low trait values, *D. ananassae* had significantly greater fractional carbohydrate content than all other species, and *D. malerkotliana* was intermediate between *D. ananassae* and the other three species (figure 6).

Desiccation resistance

Both absolute desiccation resistance and desiccation resistance per microgram carbohydrate varied significantly across species, with significant ANOVA main effects of species and sex (table 4). The species × sex interaction, however, was significant only for desiccation resistance per microgram carbohydrate (table 4). Mean absolute desiccation resistance of females (22.89 h) was significantly greater than that of males (20.86 h), whereas mean desiccation resistance per microgram carbohydrate in males (1.298 h/μg) was significantly higher than in females (0.922 h/μg) (figure 7). Overall, averaged across sexes,

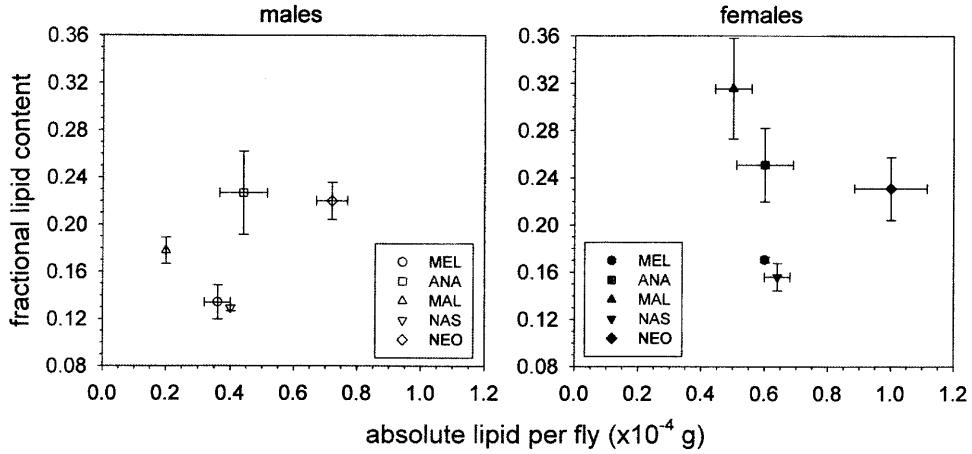

Figure 4. The relationship between mean (± SE) absolute lipid content (in 10^{-4} g per fly) and mean (± SE) fractional lipid content of males and females from the five species.

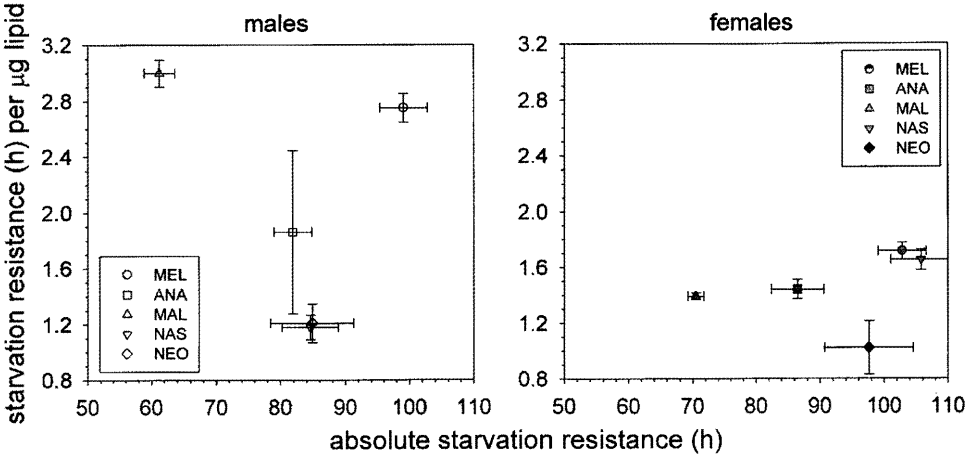

Figure 5. The relationship between mean (± SE) absolute starvation resistance (in hours) and mean (± SE) starvation resistance in hours per μg lipid of males and females from the five species.

mean absolute desiccation resistance and desiccation resistance per microgram carbohydrate of the long-term laboratory population of *D. melanogaster* were far higher than those of any of the other species (figure 7). For both males and females, all pairwise differences in absolute desiccation resistance between species were significant (Tukey's HSD, $P < 0.05$), except for those between *D. ananassae* and *D. n. nasuta*. In the case of desiccation resistance per microgram carbohydrate, all pairwise differences between species were significant (Tukey's HSD, $P < 0.05$), except for those between *D. ananassae* and *D. s. neonasuta*, for both males and females. Although *D. malerkotliana* had the least absolute desiccation resistance, its desiccation resistance per microgram carbohydrate was significantly greater than those of all other species except *D. melanogaster* (figure 7).

Discussion

In terms of fitness-related traits, the long-term laboratory population of *D. melanogaster* appears to be clearly superior under laboratory conditions to the four recently wild-caught species. *D. melanogaster* flies live longer and are more fecund on both a daily and lifetime bases than the other four species (figures 2, 3; table 5). *D. melanogaster* flies also have somewhat greater starvation resistance, and vastly greater desiccation resistance, than the four recently caught species (figures 5, 7; table 5), despite having relatively low absolute and fractional glycogen and lipid content (figures 4, 6; table 5), and being of intermediate weight (figure 1; table 5). In an earlier study on the same five populations, we found that *D. melanogaster* showed faster larval development, and greater larval survivorship and early-life fecundity, compared to the four recently caught species (Sharmila Bharathi *et al*. 2004). Of course, the superiority of the *D. melanogaster* population is not too surprising, given that this population has been in the laboratory for several hundred generations, and is presumably well adapted to the specific conditions in the laboratory. Indeed, one of the reasons for including this population in this study was to have a point of reference from a well-characterized population to act as a backdrop against which we could compare the four wild-caught

Table 4. Summary of results from four separate analyses of variance carried out on absolute carbohydrate content, fractional carbohydrate content, absolute desiccation resistance and desiccation resistance per microgram carbohydrate in males and females from the five species.

	Absolute carbohydrate content		Fractional carbohydrate content		Absolute desiccation resistance		Desiccation resistance per µg carbohydrate	
Effect (d.f.)	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species (4)	10.94	< 0.001	19.91	< 0.001	170.53	< 0.001	499.51	< 0.001
Sex (1)	19.78	< 0.001	4.02	0.052	16.28	< 0.001	226.04	< 0.001
Species × sex (4)	0.70	0.595	0.83	0.516	0.37	0.832	14.91	< 0.001

Error d.f. were 38 and 40 for the ANOVAs on carbohydrate content and desiccation resistance, respectively.

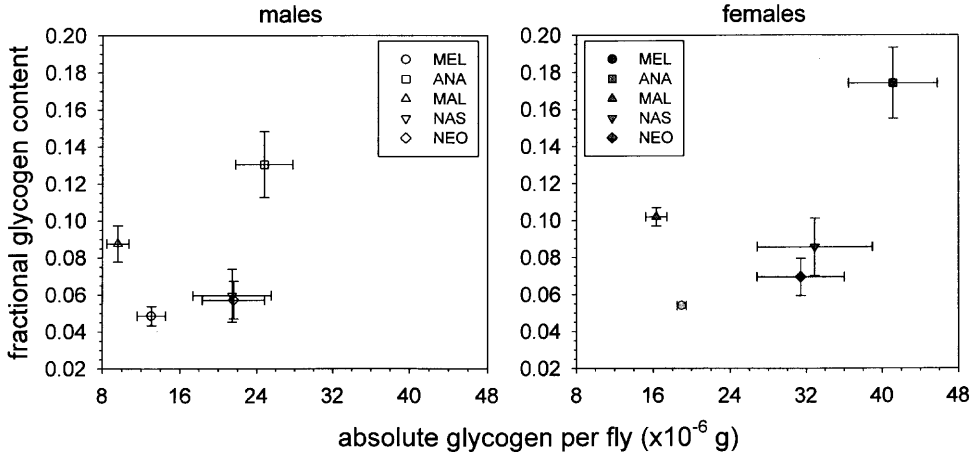

Figure 6. The relationship between mean (± SE) absolute carbohydrate content (in µg per fly) and mean (± SE) fractional carbohydrate content of males and females from the five species.

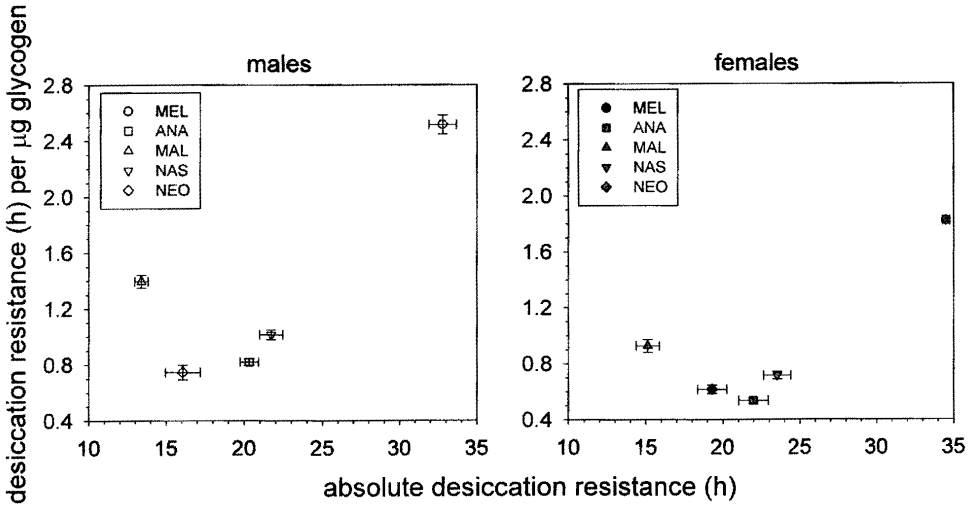

Figure 7. The relationship between mean (± SE) absolute desiccation resistance (in hours) and mean (± SE) desiccation resistance in hours per μg carbohydrate of males and females from the five species.

Table 5. A qualitative representation of how the five species compare in terms of female traits related to adult life history.

	Deviation of species mean for a trait, relative to overall mean, in units of standard error						
	> – 3	– 3 to – 2	– 2 to – 1	– 1 to + 1	+ 1 to + 2	+ 2 to + 3	> + 3
D. melanogaster		FC	AC FL	AL DW	AS LS	SL	DF DC AD
D. ananassae			DC DW	DF AD SL AS AL	FL	AC LS	FC
D. malerkotliana	AS DW	AD AC	AL	DF DC FC SL LS			FL
D. n. nasuta		FL LS		DF DC FC AD AL	AC SL DW	AS	
D. s. neonasuta	SL	DF	DC FC AD LS	AC FL AS		DW	AL

Entries are abbreviated traits (LS, lifespan of reproducing flies; DW, dry weight at eclosion; AL, absolute lipid content; AS, absolute starvation resistance; FL, fractional lipid content; SL, starvation resistance per unit lipid; AC, absolute carbohydrate content; AD, absolute desiccation resistance; FC, fractional carbohydrate content; DC, desiccation resistance per unit carbohydrate; DF, average daily fecundity). For each species, the location of the trait abbreviation reflects the deviation of the species mean from the overall mean value for that trait, in units of standard error.

species. The magnitude of *D. melanogaster* superiority, nevertheless, is rather large, and we shall return to this issue later in the discussion.

More interesting than the superiority of *D. melanogaster* is the observation that the *melanogaster* group species seem to be somewhat superior to the *immigrans* group species with regard to most of the fitness traits studied. *D. ananassae* and *D. malerkotliana*, though lighter, are longer-lived and more fecund than *D. n. nasuta* and *D. s. neonasuta* (figures 1, 2, 3; table 6), and were earlier seen to have shorter larval development time than the two *immigrans* group species (Sharmila Bharathi *et al*. 2004). On the other hand, *D. malerkotliana* has lower absolute starvation and desiccation resistance than *D. n. nasuta* and *D. s. neonasuta* (figures 5, 7; table 6), and *D. ananassae* females also have lower absolute starvation resistance than the two *immigrans* group species, although male starvation resistance and absolute desiccation resistance of *D. ananassae* is at par with that of *D. n. nasuta* and *D. s. neonasuta* (figures 5, 7; table 6). In larval survivorship, *D. ananassae* and *D. s. neonasuta* are superior to *D. malerkotliana* and *D. n. nasuta* (Sharmila Bharathi *et al*. 2004).

Why the two *melanogaster* group species appear to be somewhat superior to the two *immigrans* group species under laboratory conditions is not clear at this time. It may be that these *melanogaster* group species are in a sense preadapted to a laboratory environment optimized for *D. melanogaster*, a species to which they are closely related. It may also be that oviposition preferences differ among these four species and, thus, fecundity differences reflect degrees of behavioural aversion rather than physiological differences among species. The superiority of *D. ananassae* and *D. malerkotliana* in the laboratory does not seem to translate into greater abundance in the wild. In our collections, we found that *D. n. nasuta* and *D. ananassae*, together with *Zaprionus indianus*, were the most abundant drosophilids, with *D. ananassae* being restricted largely to the insides of houses, and *D. n. nasuta* to domestic garbage dumps and orchards, where it coexisted with, and greatly outnumbered, *D. malerkotliana*. Of course, the superiority of *D. ananassae* and, especially, *D. malerkotliana*, in terms of fecundity and lifespan is likely to be partly offset in natural conditions where desiccation and starvation stress may play a role.

Two other major aspects in which natural conditions are likely to differ from laboratory conditions are the degree of intraspecific and interspecific competition, and the presence of predators, parasites, parasitoids and microbial pathogens. Larvae from many dipteran species are found in the contents of domestic garbage dumps, whereas in rotting fruit in orchards most larvae tend to be of drosophilids. Unfortunately, there are no data on the typical larval densities likely to be experienced by these species in their natural habitats and it is, therefore, difficult to

Table 6. A qualitative representation of how the four recently wild-caught species compare in terms of female traits related to adult life history.

	Deviation of species mean for a trait, relative to overall mean, in units of standard error				
	– 3 to – 2	– 2 to – 1	– 1 to + 1	+ 1 to + 2	+ 2 to + 3
D. ananassae		DC DW	DF SL FL AS AL	AD	FC AC LS
D. malerkotliana	AD AC AS DW		FC SL AL LS		DF DC FL
D. n. nasuta	FL	LS	DF DC FC AC AL	AD DW	SL AS
D. s. neonasuta	SL DF	FC LS	DC AD AC FL AS	DW	AL

Abbreviations and interpretation as in table 5.

assess how the densities used in the laboratory compare to those to which the species are adapted. We do, however, have preliminary data suggesting that *D. ananassae* and *D. n. nasuta* differ in the sensitivity of preadult survivorship to larval density in the laboratory. Preadult survivorship of *D. ananassae* declines by just about 12.5% over a range of densities increasing from 70 to 300 eggs per vial, whereas preadult survivorship of *D. n. nasuta* declines by about 60% as density is increased from 30 to 200 eggs per vial (N. Sharmila Bharathi and A. Joshi, unpublished data), which is similar to the decline seen in our laboratory-adapted *D. melanogaster* populations (M. Shakarad, N. G. Prasad and A. Joshi, unpublished data). This may, perhaps, suggest that *D. ananassae* in nature experience higher larval densities on average than *D. n. nasuta*, an interpretation also consistent with the observation that *D. ananassae* larvae are more resistant to toxic levels of urea in the food, compared to *D. n. nasuta* larvae (N. Sharmila Bharathi and A. Joshi, unpublished data). In pairwise competition experiments where serial-transfer cultures were initiated with equal numbers of males and females of two species, *D. ananassae* excluded *D. n. nasuta* and *D. s. neonasuta* within 10 weeks (N. Sharmila Bharathi and A. Joshi, unpublished data), a finding consistent with the notion that *D. ananassae* is better adapted to high larval densities. However, the relationship between sensitivity to larval density in single-species culture and competitive ability in a serial-transfer culture, in which fecundity and lifespan also affect competition, is not so straightforward: in these experiments, our *D. melanogaster* are the most competitive, excluding all other species within 4–5 weeks. *D. malerkotliana*, which coexists with *D. n. nasuta* and is far less abundant in and around garbage dumps, appears to be a better competitor than *D. n. nasuta* and *D. s. neonasuta* under laboratory conditions, although not as good as *D. ananassae*. In serial-transfer competition experiments, *D. malerkotliana* reduced the numbers of the two *immigrans* species to 10–20% of the total culture within 10 weeks (N. Sharmila Bharathi and A. Joshi, unpublished data).

We also have some preliminary evidence that *D. n. nasuta* may be more resistant to bacterial pathogens than *D. ananassae*. Immune responses are potentially costly to the organism mounting them and are likely to exact fitness costs, especially under stressful conditions like crowding or desiccation/starvation stress (Hoang 2001; Kraaijeveld *et al*. 2001a), suggesting that parasite/pathogen loads in nature may be partly responsible for shaping the evolution of life histories (Zuk and Stoehr 2002). The immune responses mounted by *D. melanogaster* in response to infection with different types of microbial pathogens are now also quite well understood at the molecular level (reviewed by Hoffmann 2003). We have found that keeping flies in a vial with a lawn of *Escherichia coli* (Gram-negative bacterium, known to induce immune responses in *D. melanogaster*) growing on the food medium leads to fairly high levels of daily mortality, resulting in a mean time to death that is of the order of about 60–170 h, depending on the species (N. Sharmila Bharathi, J. Mohan and A. Joshi, unpublished manuscript). Both *D. n. nasuta* and *D. ananassae* have about two-fold to three-fold higher survival times in the presence of *E. coli* than the laboratory population of *D. melanogaster*, and *D. n. nasuta* survival times are significantly greater than those of *D. ananassae* (N. Sharmila Bharathi and A. Joshi, unpublished data). It is, therefore, possible that *D. n. nasuta* invests more resources in survival in a pathogen-rich environment than it does in fecundity, and that this contributes, in part, to its high abundance in the wild, compared to the other species studied.

While *D. n. nasuta* and *D. malerkotliana* are seen mating around domestic garbage dumps, in the case of *D. ananassae* it is unclear where they breed. Adult *D. ananassae* are typically found inside houses, and females can be seen ovipositing on exposed fruit in kitchens and dining rooms. None of the other species discussed here was ever seen inside a house and, conversely, *D. ananassae* is hardly ever seen outside houses, even in garbage dumps close to residential areas. Inside houses, it is more or less impossible for *D. ananassae* to complete a life cycle on either kitchen waste, or fruits, as these tend to be either thrown out, or consumed within 2–4 days. One possible hypothesis could be that *D. ananassae* oviposit on kitchen waste in houses, development is completed outside in the communal garbage dump, and the adults fly back and colonize houses afresh each generation. If true, this might be the reason for the extremely high carbohydrate content of *D. ananassae* at eclosion (figure 6), which, nevertheless, does not translate into high desiccation resistance (figure 7). Glycogen content is strongly positively correlated with flight performance (Wigglesworth 1949; Graves *et al*. 1992), and *D. ananassae* are perhaps required to fly more than the other species if they are to recolonize houses (including first-floor and second-floor houses) as young adults every generation.

All traits assayed on both males and females showed evidence for some sexual dimorphism. Across species, females had significantly greater absolute and fractional lipid and carbohydrate content, and absolute starvation and desiccation resistance than males, whereas males had significantly greater starvation resistance per microgram lipid and desiccation resistance per microgram carbohydrate than females (figures 4, 5, 6, 7; tables 3, 4). However, the species × sex interaction was significant only in the case of starvation resistance per microgram lipid and desiccation resistance per microgram carbohydrate, suggesting that the degree of dimorphism for the other traits did not differ significantly among species. In the case of adult lifespan, the degree of dimorphism was significant only in the laboratory population of *D. melanogaster*, which was also the only population in which virgins lived

significantly longer than reproducing flies (figure 2). We previously found that these five species did not differ among themselves in the degree of dimorphism for dry weight at eclosion, and that the degree of dimorphism for egg-to-eclosion development time was significantly greater than zero only in the case of *D. malerkotliana* and *D. ananassae*, and was strongly positively correlated with fecundity per day per unit dry weight (Sharmila Bharathi *et al*. 2004).

Overall, the broad picture of trait relationships across the four wild species that emerges is that of the *melanogaster* group species being more sexually dimorphic for development time (Sharmila Bharathi *et al*. 2004), more competitive (N. Sharmila Bharathi and A. Joshi, unpublished data), lighter, longer-lived, more fecund, and having higher fractional lipid and carbohydrate content than the *immigrans* group species (figures 1, 2, 3, 4, 6; table 6). The *immigrans* group species, on the other hand, tend to have higher starvation and desiccation resistance, which is not surprising given their larger size and higher absolute lipid content (figures 4, 5, 7; table 6). The negative relationship between fecundity and lifespan and the positive relationship between size and fecundity that are often seen within species are, thus, not seen across the species used in our study. To what extent the differences between the *immigrans* and *melanogaster* group species in this study are due to phylogenetic or ecological reasons is hard to assess at this time. However, there are some major differences between species within species group, suggesting that ecology may be playing a role in shaping the life history in these species.

Finally, our results on life-history and stress-related traits in these five species strike a cautionary note in the context of recent findings that wild populations of *D. melanogaster* may be considerably longer-lived and more starvation/desiccation resistant compared to long-term laboratory-reared populations, especially if kept on relatively short, discrete-generation maintenance regimes (Sgrò and Partridge 2000; Linnen *et al*. 2001; Hoffmann *et al*. 2001b). Relative to the populations of the four recently wild-caught species, our laboratory-adapted population of *D. melanogaster* is intermediate in dry weight, but the most fecund and long-lived (figures 2, 3; table 5). Both males and females of the *D. melanogaster* population have relatively low fractional lipid and glycogen content at eclosion but very high starvation resistance per microgram lipid and desiccation resistance per microgram carbohydrate, relative to the four wild-caught species (figures 4, 5, 6, 7; table 5). It is possible that the laboratory-adapted population of *D. melanogaster* has evolved to take full advantage of life in a nutrition-rich environment, and supplements a relatively lower degree of larval resource provisioning—higher larval provisioning is known to trade off with preadult survival (Chippindale *et al*. 1996, 1998)—with a relatively higher level of resource assimilation during the adult stage. However, this cannot explain the fact that even in the first few days of life, when fecundity is strongly dependent on larval reserves, *D. melanogaster* females are significantly more fecund that those of the other four species (Sharmila Bharathi *et al*. 2004). In general, although *D. melanogaster* females appear to be investing relatively more resources in reproduction—being more fecund than other species, and also the only one in which virgin females live longer than reproducing females—, they also have high starvation resistance per microgram lipid and desiccation resistance per microgram carbohydrate, suggesting that they are more efficient at using these reserves than flies of the recently wild-caught species. It is possible that our *D. melanogaster* population has evolved lower activity levels or metabolic rate, or both, in the course of several hundred generations of life in a confined environment where rich food and mates are readily available at all times. In addition, the wild-caught species may, perhaps, be investing some reserves in maintaining high levels of immune-system competence, and these reserves may, consequently, not be available for either fecundity or starvation/desiccation resistance.

While the reasons for the superior fecundity, lifespan and stress resistance of our *D. melanogaster* population are not clear at this time, our results do suggest that it may be premature to generalize about life-history contrasts between the laboratory and the field. Our comparison, of course, differs from recent studies of the laboratory–field contrast (Sgrò and Partridge 2000; Linnen *et al*. 2001; Hoffmann *et al*. 2001b) in several respects. In those studies, different populations of the same species, *D. melanogaster*, were compared, whereas we are comparing traits across species. Moreover, the populations used in the *D. melanogaster* studies are all temperate in origin, whereas we are using tropical wild populations, and a laboratory population of *D. melanogaster* that several hundred generations ago was a temperate one. The relationships between traits like starvation resistance, desiccation resistance and lipid content across species appear to be different among tropical versus temperate species (van Herrewege and David 1997). It is also possible that predators, pathogens and competitors may be more important than abiotic factors like temperature and humidity as selective forces acting on life histories in tropical drosophilids, whereas the converse may be true in temperate regions (e.g. see David and Capy 1982; Kraaijeveld and van Alphen 1995; van Herrewege and David 1997; James and Partridge 1998; Kraaijeveld *et al*. 2001b; Hoffmann *et al*. 2003). Life-history tradeoffs can be fairly labile evolutionarily (Phelan *et al*. 2003), and biotic and abiotic factors are known to interact in complex ways in their effects on community structure in dipterans (Worthen *et al*. 1994, 1995). Thus, if the balance of biotic and abiotic selective agents is very different in tropical versus temperate dro-

sophilids, it is likely that fairly different patterns of relationships among life-history-related traits will be seen in the two kinds of species. One obvious lacuna plaguing studies of life-history evolution in wild populations of *Drosophila* is the extreme paucity of knowledge about the ecology and life history of most *Drosophila* species in the wild. For example, life expectancy in the wild is clearly of great importance in interpreting differences in lifespan (assayed in the laboratory) in wild and laboratory populations. Yet, little is known about typical life expectancy of *Drosophila* in the wild. Field cage experiments suggest that life expectancy of *Drosophila* under quasinatural conditions is quite high (Hoffmann *et al*. 2003). On the other hand, a mark-and-recapture study of several temperate *Drosophila* species suggests that life expectancy in the field may actually be quite low, of the order of a few days (Rosewell and Shorrocks 1987). Unfortunately, very little is known about the ecology or life history in the wild for the four Indian species we used in this study. However, we believe that these results, though extremely preliminary, suggest that fairly different constellations of life-history-related traits have evolved in these four species, and indicate that more detailed work on the field ecology of these species could perhaps help elucidate the selection pressures that have given rise to these life-history differences. The results also permit several speculative hypotheses which can act as a spur to further experimental work on the possible role of abiotic and biotic factors, and their interactions, in shaping the life histories of these species of *Drosophila*.

Acknowledgements

We thank M. Rajamani, Sutirth Dey, K. S. Adarsh, Archan Ganguly, N. Rajanna and M. Manjesh for assistance in the laboratory, and Profs. Jean R. David and H. A. Ranganath for advice on species identification. We also thank Prof. H. A. Ranganath for supplying us with *D. s. neonasuta* flies collected from Mysore. This work was partly supported by funds from the Department of Science and Technology, Government of India. N.G.P. thanks the Council of Scientific and Industrial Research, Government of India, for financial support through a Senior Research Fellowship.

References

Chippindale A. K., Leroi A. M., Kim S. B. and Rose M. R. 1993 Phenotypic plasticity and selection in *Drosophila* life-history evolution. 1. Nutrition and the cost of reproduction. *J. Evol. Biol.* **6**, 171–193.

Chippindale A. K., Chu T. J. F. and Rose M. R. 1996 Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* **50**, 753–766.

Chippindale A. K., Leroi A. M., Saing H., Borash D. J. and Rose M. R. 1997 Phenotypic plasticity and selection in *Drosophila* life history evolution. 2. Diet, mates and the cost of reproduction. *J. Evol. Biol.* **10**, 269–293.

Chippindale A. K., Gibbs A. G., Sheik M., Yee K. J., Djawdan M., Bradley T. J. and Rose M. R. 1998 Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evolution* **52**, 1342–1352.

Cordts R. and Partridge L. 1996 Courtship reduces longevity of male *Drosophila melanogaster*. *Anim. Behav.* **52**, 269–278.

da Lage J. L., Capy P. and David J. R. 1990 Starvation and desiccation tolerance in *Drosophila melanogaster*: differences between European, North African and Afrotropical populations. *Genet. Sel. Evol.* **22**, 381–391.

David J. R. and Capy P. 1982 Genetics and origin of a *Drosophila melanogaster* population recently introduced to the Seychelles. *Genet. Res.* **40**, 295–303.

David J. R., Allemand R., van Herrewege J. and Cohet Y. 1983 Ecophysiology: abiotic factors. In *The genetics and biology of Drosophila* (ed. M Ashburner, H. L. Carson and J. N. Thompson Jr), pp. 105–170. Academic Press, London.

Djawdan M., Sugiyama T. T., Schlaeger L. K., Bradley T. J. and Rose M. R. 1996 Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. *Physiol. Zool.* **69**, 1176–1195.

Djawdan M., Rose M. R. and Bradley T. J. 1997 Does selection for stress resistance lower metabolic rate? *Ecology* **78**, 828–837.

Graves J. L., Toolson E. C., Jeong C., Vu L. N. and Rose M. R. 1992 Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiol. Zool.* **65**, 268–286.

Harshman L. G. and Hoffmann A. A. 2000 Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* **15**, 32–36.

Harshman L. G., Hoffmann A. A. and Clark A. G. 1999 Selection for starvation resistance in *Drosophila melanogaster*: physiological correlates, enzyme activities and multiple stress responses. *J. Evol. Biol.* **12**, 370–379.

Hillesheim E. and Stearns S. C. 1992 Correlated responses in life-history traits to artificial selection for body weight in *Drosophila melanogaster*. *Evolution* **46**, 745–752.

Hoang A. 2001 Immune response to parasitism reduces resistance of *Drosophila melanogaster* to desiccation and starvation. *Evolution* **55**, 2353–2358.

Hoffmann A. A. and Harshman L. G. 1999 Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity* **83**, 637–643.

Hoffmann A. A. and Parsons P. A. 1989a An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in *Drosophila*. *Biol. J. Linn. Soc.* **37**, 117–136.

Hoffmann A. A. and Parsons P. A. 1989b Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics* **122**, 837–845.

Hoffmann A. A. and Parsons P. A. 1993a Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans*. *J. Evol. Biol.* **6**, 643–657.

Hoffmann A. A. and Parsons P. A. 1993b Selection for adult desiccation resistance in *Drosophila melanogaster*: fitness components, larval resistance and stress correlations. *Biol. J. Linn. Soc.* **48**, 43–54.

Hoffmann A. A., Hallas R., Sinclair C. and Mitrovski P. 2001a Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* **55**, 1621–1630.

Hoffmann A. A., Hallas R., Sinclair C. and Partridge L. 2001b Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* **55**, 436–438.

Hoffmann A. A., Scott M., Partridge L. and Hallas R. 2003 Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. *J. Evol. Biol.* **16**, 614–623.

Hoffmann J. A. 2003 The immune response of *Drosophila*. *Nature* **426**, 33–38.

James A. C. and Partridge L. 1998 Geographic variation in competitive ability in *Drosophila melanogaster*. *Am. Nat.* **151**, 530–537.

Karan D. and Parkash R. 1998 Desiccation tolerance and starvation resistance exhibit opposite latitudinal clines in Indian geographical populations of *Drosophila kikkawai*. *Ecol. Entomol.* **23**, 391–396.

Karan D., Dahiya N., Munjal A. K., Gibert P., Moreteau B., Parkash R. and David J. R. 1998 Desiccation and starvation tolerance of adult *Drosophila*: opposite latitudinal clines in natural populations of three different species. *Evolution* **52**, 825–831.

Kraaijeveld A. R. and van Alphen J. J. M. 1995 Geographic variation in encapsulation ability of *Drosophila melanogaster* and evidence for parasitoid-specific components. *Evol. Ecol.* **9**, 10–17.

Kraaijeveld A. R., Limentani E. C. and Godfray H. C. J. 2001a Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proc. R. Soc. London* **B268**, 259–261.

Kraaijeveld A. R., Hutcheson K. A., Limentani E. C. and Godfray H. C. J. 2001b Cost of counterdefenses to host resistance in a parasitoid of *Drosophila*. *Evolution* **55**, 1815–1821.

Leroi A. M., Kim S. B. and Rose M. R. 1994 The evolution of phenotypic life-history trade-offs: an experimental study using *Drosophila melanogaster*. *Am. Nat.* **144**, 661–676.

Linnen C., Tatar M. and Promislow D. 2001 Cultural artifacts: a comparison of senescence in natural, laboratory-adapted and artificially selected lines of *Drosophila melanogaster*. *Evol. Ecol. Res.* **3**, 877–888.

Luckinbill L. S. and Clare M. J. 1985 Selection for lifespan in *Drosophila melanogaster*. *Heredity* **55**, 9–18.

Partridge L. and Andrews R. 1985 The effect of reproductive activity on the longevity of male *Drosophila melanogaster* is not caused by an acceleration of ageing. *J. Insect Physiol.* **31**, 393–395.

Partridge L. and Fowler K. 1992 Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **46**, 76–91.

Partridge L. and Fowler K. 1993 Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster*. *Evolution* **47**, 213–226.

Phelan J. P., Archer M. A., Beckman K. A., Chippindale A. K., Nusbaum T. J. and Rose M. R. 2003 Breakdown in correlations during laboratory evolution. I. Comparative analysis of *Drosophila* populations. *Evolution* **57**, 527–535.

Prasad N. G. and Joshi A. 2003 What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us? *J. Genet.* **82**, 45–76.

Robinson S. J. W., Zwaan B. and Partridge L. 2000 Starvation resistance and adult body composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution* **54**, 1819–1824.

Rose M. R. 1984 Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**, 1004–1010.

Rose M. R., Vu L. N., Park S. V. and Graves J. L. 1992 Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp. Gerontol.* **27**, 241–250.

Rosewell J. and Shorrocks B. 1987 The implication of survival rates in natural populations of *Drosophila*: capture-recapture experiments on domestic species. *Biol. J. Linn. Soc.* **32**, 373–384

Service P. M. 1987 Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **60**, 321–326.

Service P. M., Hutchinson E. W., Mackinley M. D. and Rose M. R. 1985 Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **58**, 380–389.

Sgrò C. M. and Partridge L. 1999 A delayed wave of death from reproduction in *Drosophila*. *Science* **286**, 2521–2524.

Sgrò C. M. and Partridge L. 2000 Evolutionary responses of the life-history of wild caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am. Nat.* **156**, 341–353.

Sharmila Bharathi N., Prasad N. G., Shakarad M. and Joshi A. 2004 Correlates of sexual dimorphism for dry weight and development time in five species of *Drosophila*. *J. Zool.* (in press).

Sheeba V., Madhyastha N. A. A. and Joshi A. 1998 Oviposition preference for novel versus normal food resources in laboratory populations of *Drosophila melanogaster*. *J. Biosci.* **23**, 93–100.

Simmons F. H. and Bradley T. J. 1997 An analysis of resource allocation in response to dietary yeast in *Drosophila melanogaster*. *J. Insect Physiol.* **43**, 779–788.

StatSoft, Inc. 1995 *Statistica™ Vol. I: General conventions and statistics I.* Statsoft, Inc., Tulsa, USA.

van Herrewege J. and David J. R. 1997 Starvation and desiccation tolerances in *Drosophila*: comparison of species from different climatic origins. *Ecoscience* **4**, 151–157.

van Noordwijk A. J. and de Jong G. 1986 Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137–142.

Wigglesworth V. B. 1949 The utilization of reserve substances in *Drosophila* during flight. *J. Exp. Biol.* **26**, 150–163.

Worthen W. B., Mayrose S. and Wilson R. G. 1994 Complex interactions between biotic and abiotic factors: effects on mycophagous fly communities. *Oikos* **69**, 277–286.

Worthen W. B., Bloodworth B. R. and Hobbs M. B. 1995 Habitat variability in the effects of predation and microclimate on mycophagous fly communities. *Ecography* **18**, 248–258.

Zuk M. and Stoehr A. M. 2002 Immune defense and host life history. *Am. Nat.* **160**, S9–S22.

Zwaan B. J., Bijlsma R. and Hoekstra R. F. 1991 On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to pre-adult breeding conditions. *Heredity* **66**, 29–39.

Zwaan B. J., Bijlsma R. and Hoekstra R. F. 1995 Direct selection on life span in *Drosophila melanogaster*. *Evolution* **49**, 649–659.